B. Procedures :

1. Prime a fluidics station (available from Affymetrix, Inc., Santa Clara) with 6xSSPE/0.01% Triton X-100. Also turn on a scanner (also available from Affymetrix) and create an experimental information file according to the manufacturer's instructions.

2. Remove and save hybridization solution from chip and store at -20°C.

3. Rinse chip twice with 1X MES, 0.01% Triton X-100

4. Add 300µl streptavidin solution to chip and twirl at a speed of 40 rpm on the rotisserie at room temperature for 20 min.

5. Remove stain solution and rinse 2X with 1X MES, 0.01% Triton X-100.

6. Add 300µl antibody solution to chip and twirl at a speed of 40 rpm on the rotisserie at room temperature for 20 min.

7. Remove antibody solution and rinse 2X with 1X MES, 0.01% Triton X-100.

8. Add 300µl staining solution again to chip and twirl at a speed of 40 rpm on the rotisserie at room temperature for 20 min.

9. Insert chip into fluidics station and wash 6 times at 35°C with 6X SSPE, 0.01%Triton X-100.

10. Breathe some moisture onto the chip surface and wipe clean. Insert chip into scanner.

11. Set up the scanner at settings "slowav6k" and "scan6k" at 2.27 µm pixel size with 560 nm wavelength.

12. Scan the chip.

Additional methodology useful for practicing the invention are described in Birren et al. supra. All publications and patent applications cited above are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication or patent application were specifically and individually indicated to be so incorporated by reference.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

7. Preparing Target DNA for Hybridization

1. After reducing sample complexity and optionally labelling, target DNA can be prepared for application to a chip as follows:

	Final Concentration into chip	
TMACL: 3M	177ul	5M
Tris (pH 7.8 or 8): 10 mM	3ul	1M
Triton X-100: 0.01%	3ul	1%
Herring Sperm DNA: 100ug/ml	3ul	10mg/ml
Control Oligo b948: 50pM	3ul	5nM (optional)

Labeled DNA & H_2O(~110ul) or up to 300ul final volume

The concentration of labelled DNA can range from about 0.1pM to 100pM.

2. Denature the sample at 99°C for 5 min and spin down.
3. Warm up the chip at 40°C about 20 minutes before adding the hybridization mixture.
4. Add sample into chip (vented with P-10 pipet tip).

Put chip into an oven at 40°C and rotisserie overnight at speed of 40 rpm to allow hybridization to occur.

8. Staining and Scanning a Chip

This example illustrates a procedure for detecting hybridization of sample to probes on a chip.

A. Solutions:

1. Streptavidin-phycoerythrin Solution

1ml total (300ul/chip)

470ul water
500ul 2X MES
20µl acetylated BSA(50 mg/ml)
10ul streptavidin-phycoerythrin(1mg/ml)

2. Antibody solution

1ml total (300ul/chip)

470ul water
500ul 2X MES
20ul acetylated BSA(50mg/ml)
10ul biotinylated anti-streptavidin(1mg/ml)

6. Spin briefly.

7. Add:

Boehringer Mannheim TdT (25 U/μl)	20μL
Biotin ddATP (1 mM)	20μL

8. Tap to mix.

9. Incubate at: 37°C for 90 min
99°C for 10 min
25°C for <5 min

5. Removal of repeat sequences

This example illustrates DNase digestion of human placenta DNA, denaturation and annealing of fragments.

Human Placenta DNA is digested with DNaseI as follows:

160μg Human Placenta DNA (0.08fm for the full length)
in 220μL for fragmentation [64μL DNA (2.5ug/μL), 22μL 10X buffer,
3.5μL DNaseI (0.35U), 132μL wafer]

9 μL 480mM $NaP0_4$ Buffer, pH 7.4 is added to reach final $NaP0_4$ conc. at 126mM and a volume of 301L. The sample is denatured for 5 min at 99°C. The mixture is incubated at 65°C, for 90 min to allow repeat sequence to hybridize. The mixture is then diluted to 10mM $NaP0_4$ for HPLC.

6. HPLC Hydroxyapatite Chromatography

This example illustrates use of a hydroxyapatite column to separate single stranded and double stranded DNA . This experiment used single stranded fragments, average length 60 bases, from chromosome 21 and double stranded fragments of herring sperm DNA (average length 500 bp). Both single and double stranded DNA were present at 9uM. The column was an Econo-Pac CHT-II Cartridge having a DNA capacity of 160ug. The column was loaded with DNA in 10 mM phosphate. At 10-20 mM phosphate hydroxyapatite binds both single and double stranded DNA. DNA was then eluted at a gradient from 10 mM to 1 M $NaP0_4$ Buffer, pH 7.4 over 30 min. Elution was monitored by absorbance at 260 nm. At 5 min, there was a small peak indicating release of single stranded DNA, and at 25 min there was a larger peak indicating release of double stranded DNA, as shown in Fig. 1.

Make up volume to 74ul

2. Incubate at: 37°C for 10 min
   99°C for 10 min
   Place on ice for 2 min

3. Spin briefly.
6. Add: 2 µl of Boehringer Mannheim or BRL TdT (25 U/µl)
   1 µl of biotin ddATP (1 mM)
7. Pipet to mix.
8. Incubate at: 37°C for 90 min
   99°C for 10 min
   25°C indefinitely.

3. Fragmentation and labeling of PCR fragments

This example illustrates an alternative procedure for fragmenting by DNaseI digestion and labelling that is particularly suitable for use with long range PCR products.

1. Start with purified Long Range PCR products in final volume of 300-350uL. The concentration of DNA is determined by OD 260 measurement.
2. 280 µg DNA can be labelled to give a final target concentration of 5-10pM for a complexity range of 3-6 MB.
3. The labeling is performed in 5 independent Eppenderf tubes with each one containing:

10X One-Phor-All Buffer PLUS 37µL

Gibco DNaseI (at 0.5U/uL; 2uL
1uL Dnase 1 + 1uL
10mM Tris, pH 8)

Purified LR-PCR products up to 331uL

Total reaction volume (5Mb DNA need 280ug): 370uL

4. Tap to mix.
5. Incubate at: 37°C for 10 min
   99°C for 10 min
   25°C for <5 min

2. Pick colony with a pipet tip and shoot the entire tip into 5 ml LB with antibiotics (25 ug/ml kanamycin for PAC and 25ug/ml chloramphenicol for BAC). Put in a shaking incubator at 300 rpm overnight @ 37Co

3. Centrifuge (SM24 Rotor) at 3,000 rpm for 10 minute in the Sorvall.

4. Discard supernatants. Add 1.250 ml of Qiagen buffer "P1" to sample. Resuspend each pellet by pipetting up and down several times until no cell clumps remain.

5. Add 1.250 ml of Qiagen buffer "P2" to sample and gently shake tube to mix the contents. Let sit at room temperature for 5 min. The appearance of the suspension should change from very turbid to almost translucent.

6. Add 1.250 ml of Qiagen buffer "P3" to each tube and gently shake during addition. A thick white precipitate of protein and E. coli DNA will form. Place the tubes on ice for at least 5 min.

7. Spin sample at 10,000 rpm for 10 minutes at 4°C in the SM24 rotor.

8. Remove tubes from centrifuge and place on ice. Transfer the supernatant using a disposable pipette into a new tube and precipitate the DNA with 2 volumes of ethanol. Mix by inverting tube a few times; place tubes on ice for at least 5 minutes.

9. Spin sample at 10,000 rpm for 10 min at 4°C in the SM24 rotor.

10. Carefully pour off the ethanol. Wash the pellet with 75% ethanol and centrifuge at 10,000 rpm for 5 minute twice.

11. Place the tubes upside down on a paper towel to let the ethanol wick off the sides of the tube. Let air dry for 5-10 min until the DNA pellets turn from white to translucent in appearance that means most of the ethanol has evaporated.

12. Add 250 µl TE or water into DNA sample, allow the solution to sit in the tube on ice for about 30 minutes.

13. Determine DNA concentration by using both UV spectrophotometry and quantitative analysis on an agarose gel.

2. Fragmentation and labeling:

This example illustrates how to fragment genomic DNA by DNaseI digestion, and how to label the resulting fragments with biotin.

1. Put 30 ug BAC or PAC DNA:

   2ul of diluted DNaseI (0.1U/ul, 10 times dilution)

   7.4 10X One-Phor-All Buffer PLUS

denatured first and second tag strands are then hybridized to the complementary regions of the probes, using standard conditions described in WO 97/27317. The hybridization pattern indicates which probes are complementary to tag strands in the sample. Comparison of the hybridization pattern of two samples indicates which probes hybridize to tag strands that derive from mRNAs that are differentially expressed between the two samples. These probes are of particular interest, because they contain complementary sequence to mRNA species subject to differential expression. The sequence of such probes is known and can be compared with sequences in databases to determine the identity of the full-length mRNAs subject to differential expression provided that such mRNAs have previously been sequenced. Alternatively, the sequences of probes can be used to design hybridization probes or primers for cloning the differentially expressed mRNAs. The differentially expressed mRNAs are typically cloned from the sample in which the mRNA of interest was expressed at the highest level. In some methods, database comparisons or cloning is facilitated by provision of additional sequence information beyond that inferable from probe sequence by template dependent extension as described above.

Examples

1. Preparation DNA from BAC & PAC Clone

This example provides a method of preparing genomic DNA suitable for use in any of the methods of reducing sample complexity described above that involves use of genomic DNA.

Reagents Used:

1. Qiagen Large-Construct Kit (cat.# 12462)

2. Molecular Biology Grade Water from Bio Whittaker, Cat.# 16-001Y

3. Ethanol (Rossville Gold Shield 200 proof)

4. LB Broth Base (Gibco BRL cat # 12780-052) 20 grams per liter of DI water will give 1L of LB.

5. Bacto-Agar (Difco cat # 0140-01) 15 grams per 1L of LB.

6. Antibiotics: Chloramphenicol and Kanamycine (25ug/ml)

Protocol:

1. Streak some of the "stab" clone to a plate of LB agar containing 25 μg/ml chloramphenicol or kanamycin. Grow at 37°C for overnight.

the overexpression of a particular marker such as the HER2 (c-erbB-2/neu) protooncogene in the case of breast cancer.

Expression monitoring can be used to monitor expression of various genes in response to defined stimuli, such as a drug. This is especially useful in drug research if the end point description is a complex one, not simply asking if one particular gene is overexpressed or underexpressed. Therefore, where a disease state or the mode of action of a drug is not well characterized, the expression monitoring can allow rapid determination of the particularly relevant genes.

In arrays of random probes (sometimes known as generic arrays), the hybridization pattern is also a measure of the presence and abundance of relative mRNAs in a sample, although it is not immediately known, which probes correspond to which mRNAs in the sample. However the lack of knowledge regarding the particular genes does not prevent identification of useful therapeutics. For example, if the hybridization pattern on a particular generic array for a healthy cell is known and significantly different from the pattern for a diseased cell, then libraries of compounds can be screened for those that cause the pattern for a diseased cell to become like that for the healthy cell. This provides a detailed measure of the cellular response to a drug.

Generic arrays can also provide a powerful tool for gene discovery and for elucidating mechanisms underlying complex cellular responses to various stimuli. For example, generic arrays can be used for expression fingerprinting. Suppose it is found that the mRNA from a certain cell type displays a distinct overall hybridization pattern that is different under different conditions (e.g., when harboring mutations in particular genes, in a disease state). Then this pattern of expression (an expression fingerprint), if reproducible and clearly differentiable in the different cases can be used as a very detailed diagnostic. It is not required that the pattern be fully interpretable, but just that it is specific for a particular cell state (and preferably of diagnostic and/or prognostic relevance).

Both customized and generic arrays can be used in drug safety studies. For example, if one is making a new antibiotic, then it should not significantly affect the expression profile for mammalian cells. The hybridization pattern can be used as a detailed measure of the effect of a drug on cells, for example, as a toxicological screen.

The sequence information provided by the hybridization pattern of a generic array can be used to identify genes encoding mRNAs hybridized to an array. Such methods can be performed using DNA tags of the invention as the target nucleic acids described in WO 97/27317. DNA tags can be denatured forming first and second tag strands. The

part of the child's genotype not attributable to the mother is consistent with that of the putative father. Paternity testing can be performed by analyzing sets of polymorphisms in the putative father and the child.

If the set of polymorphisms in the child attributable to the father does not match the putative father, it can be concluded, barring experimental error, that the putative father is not the real father. If the set of polymorphisms in the child attributable to the father does match the set of polymorphisms of the putative father, a statistical calculation can be performed to determine the probability of coincidental match.

The probability of parentage exclusion (representing the probability that a random male will have a polymorphic form at a given polymorphic site that makes him incompatible as the father) is given by the equation (see WO 95/12607):

$$p(exc) = xy(1-xy)$$

where x and y are the population frequencies of alleles A and B of a diallelic polymorphic site.

(At a triallelic site $p(exc) = xy(1-xy) + yz(1- yz) + xz(1-xz)+ 3xyz(1-xyz)$)), where x, y and z and the respective population frequencies of alleles A, B and C).

The probability of non-exclusion is

$$p(non\text{-}exc) = 1-p(exc)$$

The cumulative probability of non-exclusion (representing the value obtained when n loci are used) is thus:

$$cum\ p(non\text{-}exc) = p(non\text{-}exc1)p(non\text{-}exc2)p(non\text{-}exc3)....\ p(non\text{-}excn)$$

The cumulative probability of exclusion for n loci (representing the probability that a random male will be excluded)

$$cum\ p(exc) = 1 - cum\ p(non\text{-}exc).$$

If several polymorphic loci are included in the analysis, the cumulative probability of exclusion of a random male is very high. This probability can be taken into account in assessing the liability of a putative father whose polymorphic marker set matches the child's polymorphic marker set attributable to his/her father.

2. Expression Analysis

The quantitative monitoring of expression levels for large numbers of genes can prove valuable in elucidating gene function, exploring the causes and mechanisms of disease, and for the discovery of potential therapeutic and diagnostic targets. Expression monitoring can be used to monitor the expression (transcription) levels of nucleic acids whose expression is altered in a disease state. For example, a cancer can be characterized by

organism with frequencies x and y, the probability of each genotype in a diploid organism are (see WO 95/12607):

Homozygote: p(AA)= x2

Homozygote: p(BB)= y2 = (1-x)2

Single Heterozygote: p(AB)= p(BA)= xy = x(1-x)

Both Heterozygotes: p(AB+BA)= 2xy = 2x(1-x)

The probability of identity at one locus (i.e., the probability that two individuals, picked at random from a population will have identical polymorphic forms at a given locus) is given by the equation:

p(ID) = (x2)2 + (2xy)2 + (y2)2.

These calculations can be extended for any number of polymorphic forms at a given locus. For example, the probability of identity p(ID) for a 3-allele system where the alleles have the frequencies in the population of x, y and z, respectively, is equal to the sum of the squares of the genotype frequencies:

p(ID) = x4 + (2xy)2 + (2yz)2 + (2xz)2 + z4 + y4

In a locus of n alleles, the appropriate binomial expansion is used to calculate p(ID) and p(exc).

The cumulative probability of identity (cum p(ID)) for each of multiple unlinked loci is determined by multiplying the probabilities provided by each locus.

cum p(ID) = p(ID1)p(ID2)p(ID3).... p(IDn)

The cumulative probability of non-identity for n loci (i.e. the probability that two random individuals will be different at 1 or more loci) is given by the equation:

cum p(nonID) = 1-cum p(ID).

If several polymorphic loci are tested, the cumulative probability of non-identity for random individuals becomes very high (e.g., one billion to one). Such probabilities can be taken into account together with other evidence in determining the guilt or innocence of the suspect.

B. Paternity Testing

The object of paternity testing is usually to determine whether a male is the father of a child. In most cases, the mother of the child is known and thus, the mother's contribution to the child's genotype can be traced. Paternity testing investigates whether the

Yijkpn= μ + YSi + Pj + Xk + β1 + ... β17 + PEn + an +ep where Yijknp is the milk, fat, fat percentage, SNF, SNF percentage, energy concentration, or lactation energy record; μ is an overall mean; YSi is the effect common to all cows calving in year-season; Xk is the effect common to cows in either the high or average selection line; β1 to β17 are the binomial regressions of production record on mtDNA D-loop sequence polymorphisms; PEn is permanent environmental effect common to all records of cow n; an is effect of animal n and is composed of the additive genetic contribution of sire and dam breeding values and a Mendelian sampling effect; and ep is a random residual. It was found that eleven of seventeen polymorphisms tested influenced at least one production trait. Bovines having the best polymorphic forms for milk production at these eleven loci are used as parents for breeding the next generation of the herd.

b. Forensics

Determination of which polymorphic forms occupy a set of polymorphic sites in an individual identifies a set of polymorphic forms that distinguishes the individual. See generally National Research Council, The Evaluation of Forensic DNA Evidence (Eds. Pollard et al., National Academy Press, DC, 1996). The more sites that are analyzed the lower the probability that the set of polymorphic forms in one individual is the same as that in an unrelated individual.

The capacity to identify a distinguishing or unique set of forensic markers in an individual is useful for forensic analysis. For example, one can determine whether a blood sample from a suspect matches a blood or other tissue sample from a crime scene by determining whether the set of polymorphic forms occupying selected polymorphic sites is the same in the suspect and the sample. If the set of polymorphic markers does not match between a suspect and a sample, it can be concluded (barring experimental error) that the suspect was not the source of the sample. If the set of markers does match, one can conclude that the DNA from the suspect is consistent with that found at the crime scene. If frequencies of the polymorphic forms at the loci tested have been determined (e.g., by analysis of a suitable population of individuals), one can perform a statistical analysis to determine the probability that a match of suspect and crime scene sample would occur by chance.

p(ID) is the probability that two random individuals have the same polymorphic or allelic form at a given polymorphic site. In diallelic loci, four genotypes are possible: AA, AB, BA, and BB. If alleles A and B occur in a haploid genome of the

Correlation is performed for a population of individuals who have been tested for the presence or absence of one or more phenotypic traits of interest and for polymorphic profile. The alleles of each polymorphism in the profile are then reviewed to determine whether the presence or absence of a particular allele is associated with the trait of interest. Correlation can be performed by standard statistical methods such as a κ-squared test and statistically significant correlations between polymorphic form(s) and phenotypic characteristics are noted. For example, it might be found that the presence of allele A1 at polymorphism A correlates with heart disease. As a further example, it might be found that the combined presence of allele A1 at polymorphism A and allele B1 at polymorphism B correlates with increased risk of cancer.

Such correlations can be exploited in several ways. In the case of a strong correlation between a set of one or more polymorphic forms and a disease for which treatment is available, detection of the polymorphic form set in a human or animal patient may justify immediate administration of treatment, or at least the institution of regular monitoring of the patient. Detection of a polymorphic form(s) correlated with serious disease in a couple contemplating a family may also be valuable to the couple in their reproductive decisions. For example, the female partner might elect to undergo in vitro fertilization to avoid the possibility of transmitting such a polymorphism from her husband to her offspring. In the case of a weaker, but still statistically significant correlation between a polymorphic set and human disease, immediate therapeutic intervention or monitoring may not be justified. Nevertheless, the patient can be motivated to begin simple life-style changes (e.g., diet, exercise) that can be accomplished at little cost to the patient but confer potential benefits in reducing the risk of conditions to which the patient may have increased susceptibility by virtue of variant alleles. Identification of a polymorphic profiles in a patient correlated with enhanced receptiveness to one of several treatment regimes for a disease indicates that this treatment regime should be followed.

For animals and plants, correlations between polymorphic profiles and phenotype are useful for breeding for desired characteristics. For example, Beitz et al., US 5,292,639 discuss use of bovine mitochondrial polymorphisms in a breeding program to improve milk production in cows. To evaluate the effect of mtDNA D-loop sequence polymorphism on milk production, each cow was assigned a value of 1 if variant or 0 if wildtype with respect to a prototypical mitochondrial DNA sequence at each of 17 locations considered. Each production trait was analyzed individually with the following animal model:

amplification methods involve isothermal reactions based on isothermal transcription, which produce both single stranded RNA (ssRNA) and double stranded DNA (dsDNA) as the amplification products in a ratio of about 30 or 100 to 1, respectively.

V. Uses of Genetic Analyses

1. Polymorphic profiling

a. Association Studies and Diagnosis

The polymorphic profile of an individual may contribute to phenotype of the individual in different ways. Some polymorphisms occur within a protein coding sequence and contribute to phenotype by affecting protein structure. The effect may be neutral, beneficial or detrimental, or both beneficial and detrimental, depending on the circumstances. For example, a heterozygous sickle cell mutation confers resistance to malaria, but a homozygous sickle cell mutation is usually lethal. Other polymorphisms occur in noncoding regions but may exert phenotypic effects indirectly via influence on replication, transcription, and translation. A single polymorphism may affect more than one phenotypic trait. Likewise, a single phenotypic trait may be affected by polymorphisms in different genes. Further, some polymorphisms predispose an individual to a distinct mutation that is causally related to a certain phenotype.

Phenotypic traits include diseases that have known but hitherto unmapped genetic components (e.g., agammaglobulimenia, diabetes insipidus, Lesch-Nyhan syndrome, muscular dystrophy, Wiskott-Aldrich syndrome, Fabry's disease, familial hypercholesterolemia, polycystic kidney disease, hereditary spherocytosis, von Willebrand's disease, tuberous sclerosis, hereditary hemorrhagic telangiectasia, familial colonic polyposis, Ehlers-Danlos syndrome, osteogenesis imperfecta, and acute intermittent porphyria). Phenotypic traits also include symptoms of, or susceptibility to, multifactorial diseases of which a component is, or may be, genetic, such as autoimmune diseases, inflammation, cancer, diseases of the nervous system, and infection by pathogenic microorganisms. Some examples of autoimmune diseases include rheumatoid arthritis, multiple sclerosis, diabetes (insulin-dependent and non-independent), systemic lupus erythematosus and Graves disease. Some examples of cancers include cancers of the bladder, brain, breast, colon, esophagus, kidney, leukemia, liver, lung, oral cavity, ovary, pancreas, prostate, skin, stomach and uterus. Phenotypic traits also include characteristics such as longevity, appearance (e.g., baldness, obesity), strength, speed, endurance, fertility, and susceptibility or receptivity to particular drugs or therapeutic treatments.

4. Nucleic Acid Sample Preparation

The nucleic acid samples hybridized to arrays can be genomic, RNA or cDNA. Optionally, nucleic acid samples can be subject to amplification before or after enrichment.. An individual genomic DNA segment from the same genomic location as a designated reference sequence can be amplified by using primers flanking the reference sequence. Multiple genomic segments corresponding to multiple reference sequences can be prepared by multiplex amplification including primer pairs flanking each reference sequence in the amplification mix. Alternatively, the entire genome can be amplified using random primers (typically hexamers) (see Barrett et al., Nucleic Acids Research 23, 3488-3492 (1995)) or by fragmentation and reassembly (see, e.g., Stemmer et al., Gene 164, 49-53 (1995)). Genomic DNA can be obtained from virtually any tissue source (other than pure red blood cells). For example, convenient tissue samples include whole blood, semen, saliva, tears, urine, fecal material, sweat, buccal, skin and hair. RNA samples are also often subject to amplification. In this case amplification is typically preceded by reverse transcription. Amplification of all expressed mRNA can be performed, for example, as described by commonly owned WO 96/14839 and WO 97/01603

5. Methods of amplification

The PCR method of amplification is described in PCR Technology: Principles and Applications for DNA Amplification (ed. H.A. Erlich, Freeman Press, NY, NY, 1992); PCR Protocols: A Guide to Methods and Applications (eds. Innis, et al., Academic Press, San Diego, CA, 1990); Mattila et al., Nucleic Acids Res. 19, 4967 (1991); Eckert et al., PCR Methods and Applications 1, 17 (1991); PCR (eds. McPherson et al., IRL Press, Oxford); and U.S. Patent 4,683,202 (each of which is incorporated by reference for all purposes). Nucleic acids in a target sample can be labelled in the course of amplification by inclusion of one or more labelled nucleotides in the amplification mix. Labels can also be attached to amplification products after amplification e.g., by end-labelling. The amplification product can be RNA or DNA depending on the enzyme and substrates used in the amplification reaction.

Other suitable amplification methods include the ligase chain reaction (LCR) (see Wu and Wallace, Genomics 4, 560 (1989), Landegren et al., Science 241, 1077 (1988), transcription amplification (Kwoh et al., Proc. Natl. Acad. Sci. USA 86, 1173 (1989)), and self-sustained sequence replication (Guatelli et al., Proc. Nat. Acad. Sci. USA, 87, 1874 (1990)) and nucleic acid based sequence amplification (NASBA). The latter two

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **METHODS FOR REDUCING COMPLEXITY OF NUCLEIC ACID SAMPLES** the specification of which is attached hereto.

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56. I claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Country	Application No.	Date of Filing	Priority Claimed Under 35 USC 119

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date
60/228,251	August 26,2000

I claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Date of Filing	Status

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 1	Signature of Inventor 2
Nila Patil	David Cox
Date	Date

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